ORIGINAL ARTICLE MYCOLOGY

Survival of influenza virus on human fingers

Y. Thomas[1], P. Boquete-Suter[1], D. Koch[2], D. Pittet[3] and L. Kaiser[1]

1) *Laboratory of Virology and Swiss National Reference Center for Influenza, Division of Infectious Diseases and Division of Laboratory Medicine, University Hospitals of Geneva and Medical School, University of Geneva, Geneva,* 2) *Division of Communicable Diseases, Federal Office of Public Health, Bern* and 3) *Infection Control Programme and WHO Collaborating Centre on Patient Safety, University Hospitals of Geneva and Medical School, University of Geneva, Geneva, Switzerland*

Abstract

Indirect transmission of the influenza virus via finger contamination with respiratory mucus droplets has been hypothesized to contribute to transmission in the community. Under laboratory conditions, influenza-infected respiratory droplets were reconstituted as close as possible to natural conditions. We investigated experimentally the survival of influenza A (H3N2) and A (H1N1)pdm09 viruses on human fingers. Infectious virus was easily recoverable on all fingers 1 min after fingertip contamination but then decreased very rapidly. After 30 min, infectious virus was detectable in only a small minority of subjects. Infectious viruses were detected for a longer period of time when droplets of larger size containing a higher number of particles were tested or when the viral concentration increased. A rapid decrease in infectiousness was observed when droplet integrity was disrupted. Our findings could help to set up the promotion of hand hygiene to prevent influenza hand contamination.

Keywords: Fingers, H1N1 2009, influenza, transmission, viral stability
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Corresponding author: Y. Thomas, Laboratory of Virology and Swiss National Reference Center of Influenza, Division of Infectious Diseases and Division of Laboratory Medicine, University Hospitals of Geneva, 4 Rue Gabrielle Perret-Gentil, 1211 Geneva 14, Switzerland
E-mail: yves.thomas@hcuge.ch

Introduction

Human-to-human influenza transmission is mediated mainly by the airborne route [1,2], but direct contact via previously contaminated hands followed by self-inoculation of the upper respiratory tract is possibly equally important [3,4]. During the recent influenza A (H1N1) pandemic, this led to the inclusion of hand hygiene as one of the recommended first-line preventive measures against transmission [5,6]. However, it remains difficult to establish whether large contaminated respiratory droplets could lead to finger contamination [7,8]. This may be dependent upon the virus type, inoculum size and external conditions, such as temperature and humidity [7]. To the best of our knowledge, the role of hand contamination followed by self-inoculation is supported only by indirect epidemiological data [9] linked to the ability of the influenza virus to survive for a prolonged period under various environmental conditions, but no direct proof or human experimental studies are available.

Indirect evidence is the ability of human influenza to survive for a prolonged period of time under various environmental conditions. Previous studies performed in the 1940s and in the 1980s reported that the influenza virus preserved its infectiousness when mixed in mucin-enriched buffer [10] or conserved on dry surfaces for 5 weeks [11]. Similarly, more recently the influenza A (H3N2) virus was cultivable for up to 17 days after deposition on banknotes in the presence of respiratory mucus [12]. Avian influenza viruses remained infectious for more than 300 days at 4°C and more than 100 days at 26°C in water of appropriate acidity and salinity [13,14]. The influenza A (H1N1)pdm09 virus can potentially retain its infectivity on a non-porous surface for up to 7 days at 35°C and 66 days at 4°C [15], although other studies showed a much shorter survival time of 4–9 h [16] at room temperature. Under non-physiological laboratory conditions, large doses (1 mL) of seasonal influenza A (H1N1) culture supernatant at a high concentration (10^7 $TCID_{50}$/0.1 mL) remained infectious for 1 h on hands [17]. Closer to real-life conditions, fomites sampled in nursing homes, daycare centres or households during an influenza epidemic were contaminated with the influenza genome [18,19]. During the 2009 epidemic, nucleic acid of the influenza virus genome was detected on 17% of the fingertips of children living in the same household as confirmed influenza cases [19]. Under experimental conditions, 0.1 mL of an influenza A (H1N1) viral suspension at physiological concentration (10^3—$10^{4.5}$ $TCID_{50}$/0.1 mL) present on a non-porous support could be transferred to hands and remained infectious for several minutes [11]. However, it remains unknown whether such an inoculum could initiate transmission once in contact with the upper respiratory tract. The objective of this study was to investigate the survival rate of influenza A (H1N1) 2009 and seasonal influenza A (H3N2) virus on human fingers experimentally contaminated with reconstituted respiratory droplets as close as possible to those in infected humans.

Methods

We conducted a series of experiments to assess the survival and duration of infectiousness of human influenza viruses on human fingers between 1 and 30 min. The term 'survival' is defined as the persistence of influenza virus that could be propagated on Madin-Darby canine kidney cells (MDCK).

Cell line and conditions

MDCK cells (#CCL34™; ATCC, Manassas, VA , USA) [12] were either cultivated under serum-containing conditions in MEM-Eagle medium (DMEM, M4655-500; Sigma Chemie, Buchs, Switzerland), supplemented with 2 mg/L Trypsin (#25090028; Invitrogen/Gibco, Basel, Switzerland), 10% fetal calf serum (#10270-106; Invitrogen/Gibco) and 7.5% NaHCO 3 (#530F00H; Bio Concept, Allschwil, Switzerland), or under serum-free conditions in DMEM (#31966-021; Invitrogen/Gibco).

Viral suspensions

All experiments were performed with the vaccine strain influenza A/Moscow/10/1999 (H3N2) and the influenza A/Switzerland/01/2009 (H1N1) strain antigenically and genetically related to the vaccine strain influenza A/California/7/2009 (H1N1). One millilitre of viral suspension obtained by cell culture was mixed in human respiratory mucus. Briefly, 1 mL of cell suspension obtained by cell culture was mixed with 9 mL of human secretions, resulting in stock concentrations of 1.8×10^7 and 1×10^5 50% tissue culture infective doses ($TCID_{50}$/mL), respectively. Respiratory mucus was obtained by mixing clinical specimens received at the laboratory for respiratory virus testing by real-time RT-PCR and cell culture and which were negative.

Participants and finger contamination procedure

The recruited volunteers were six specialized laboratory collaborators (technicians, MD or PhD graduates) accustomed to infectious virus manipulation and who had undergone vaccination with the 2008–2009, 2009–2010 and pandemic 2009 influenza vaccines. Experiments were conducted on each participant on different days but with identical temperature, humidity and hood flow conditions. The study protocol was approved by the University Hospitals of Geneva ethics committee. All participants signed an informed consent form and were instructed to follow biosafety guidelines under the close supervision of the main investigator.

Hands were strictly kept under biosafety level 2 (BSL2) hoods during the complete procedure. To avoid detergent action on cell culture, hands were not washed before the experiments. At the end of each experimental procedure under the BSL2 hood, volunteers' fingers were systematically immerged in 1% bleach for 1 min before being dried and immediately re-disinfected abundantly with alcohol-based hand gel. Hands were then removed from the hood and washed again with disinfectant soap, followed by cleansing with alcohol-based gel. During the experiments the average temperature and humidity rate were maintained at 22 ± 3°C and 66 ± 5%, respectively. A 2-μL drop of influenza A (H3N2) and A (H1N1) 2009 viral suspension mixed with respiratory secretions (as described in the Viral suspension section above) was deposited on fingertips. This volume was chosen because it represents a good mean of large respiratory droplets size and can be applied in a reproducible manner (Fig. 1). Each individual contaminated finger was kept at room temperature without any contact or any additional mechanical action on the infectious suspension for 1, 3, 5, 15 and 30 min, before testing for the presence of infectious virions. Each viral suspension was deposited on three fingers of each volunteer for a total of 18 fingers. For experiments where contaminant droplets were specifically disrupted, the tip of the pipette was used to spread the droplet immediately on to the surface of the fingertip.

Determination of infectiousness

After a predefined time, the volunteers' fingers were immerged in wells containing 1 mL of Eagle minimal essential medium

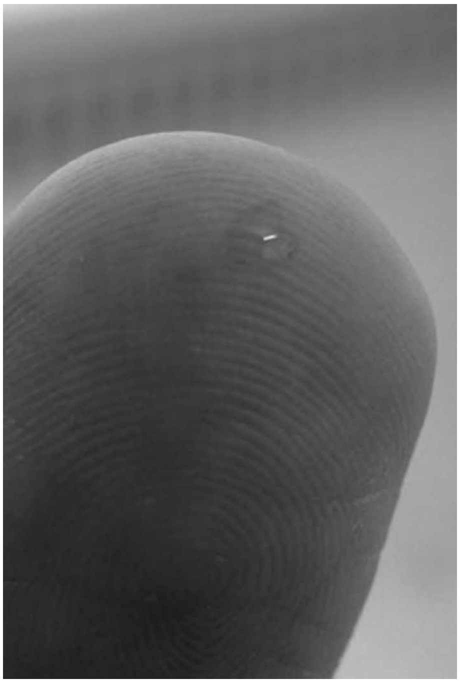

FIG 1. Fingers contaminated with a 2-μL drop of viral suspension mixed in human respiratory secretion.

supplemented with 25 mM HEPES (MEM-Eagle) for 60 s to immerge viral particles. A 0.4-mL portion of the eluate was then used immediately to inoculate MDCK cells. After 1 h adsorption at 37°C, 1 mL of serum-free MEM-Eagle medium supplemented with 2 mg/L Trypsin (T1426; Sigma-Aldrich, Buchs, Switzerland) was added and cells were incubated at 37°C and in 5% CO_2 for 7 days. For each 12-well plate, a negative control and a mock infected control finger was included. The cytopathic effect was read daily by microscope and cells were collected after 7 days' incubation by mechanical disruption, fixed after centrifugation and submitted to immunofluorescence analysis. After washing with phosphate buffered saline (PBS), cells were fixed for 20 min in acetone at −20°C. A mouse monoclonal antibody specific to the nucleoprotein of influenza A viruses and a mouse monoclonal IgG FITC conjugate antibody were used to confirm the presence of viral protein (no. 5001 and 5008; Chemicon-Millipore, Zug, Switzerland). Positive samples were determined with a positive immunofluorescence test.

Results

After deposition on fingertips of influenza A (H3N2) and A (H1N1) 2009 viruses in reconstituted influenza-infected respiratory droplets, the viral recovery rate was determined after different time periods for each contaminated finger and individual (Fig. 2). After 1 min it was possible to recover infectious A (H1N1) 2009 and A (H3N2) viruses on all (18/18) fingers of all individuals (6/6). The proportion of fingers with recoverable infectious viral particles then declined rapidly. For influenza A (H3N2) virus-contaminated fingers, we observed a sharp decline in infectious virion recovery after 3 min (6/18 fingers remained positive). This loss was less important for A

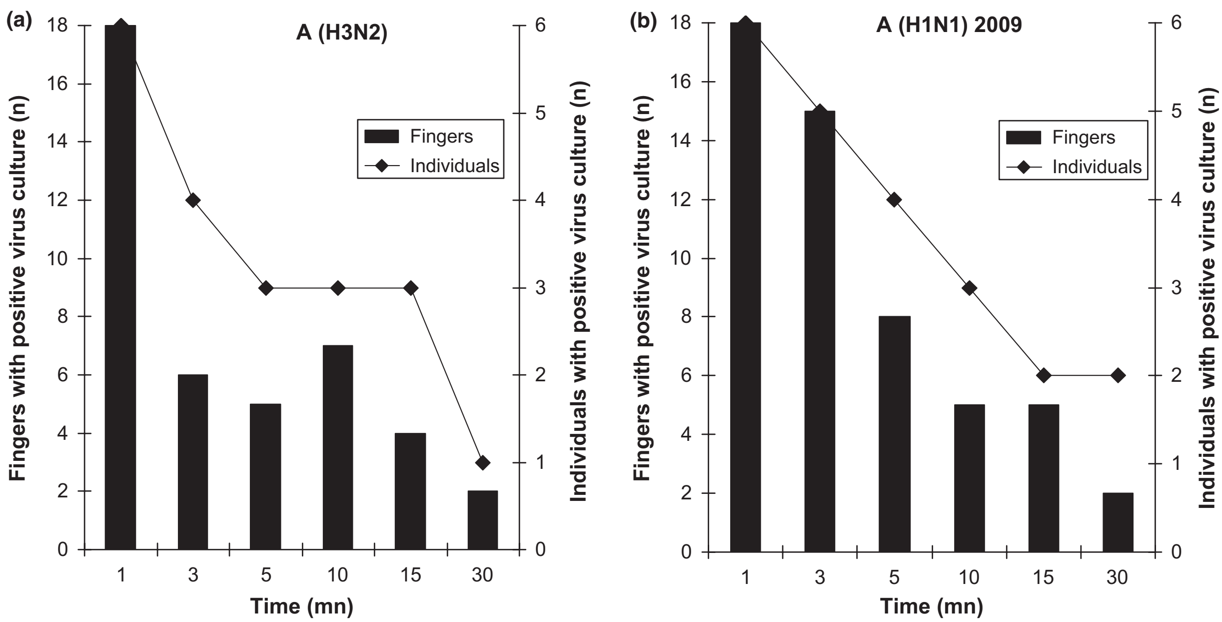

FIG 2. Influenza A (H3N2) and A (H1N1) 2009 viral survival on fingers over time (X axis). Bars (left scale) and lines (right scale) represent the absolute number of fingers and individuals, respectively, from whom infectious virus was recovered.

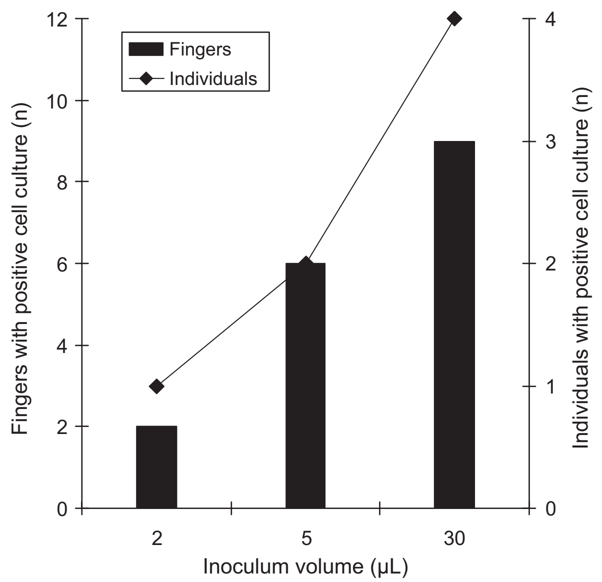

FIG 3. Influenza A (H3N2) viral survival on fingers according to the volume of the contaminated droplet. Four individuals (three fingers each) participated in this experiment, in which viral concentration was fixed. The number of fingers (bars) and individuals (curves) from whom infectious virus could be isolated after 15 min is represented according to the volume of the contaminated droplet (X axis).

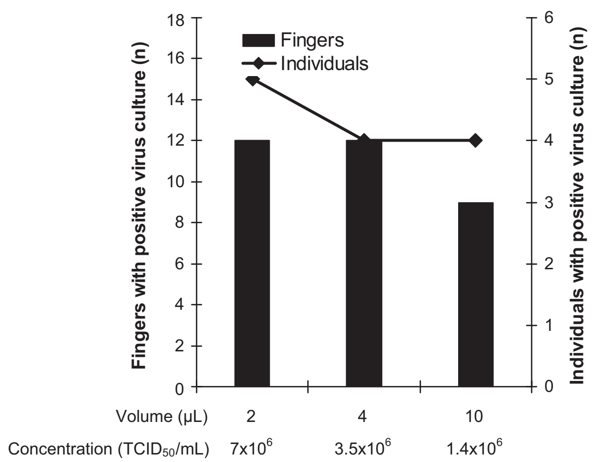

FIG 4. Influenza A (H3N2) viral survival on fingers according to volume and concentration of the contaminated droplet, each containing the same number of infectious viral particles. Six individuals (three fingers each) participated in this experiment. The number of fingers (bars) and individuals (curves) from whom infectious virus could be isolated after 15 min is represented according to the volume and the viral concentration of the contaminated droplet (X axis).

(H1N1) 2009 virus (15/18 positive fingers), despite a lower initial viral concentration of the inoculum. The positivity rate declined slowly until 15 min with 4/18 and 5/18 fingers still infectious with influenza A (H3N2) and A (H1N1) 2009 viruses, respectively. After 30 min, the number of positive fingers dropped to 2/18 fingers for each viral type.

We then performed a series of experiments to investigate to what extent viral concentration and the number of particles contained within a droplet affects influenza virus survival. In a first experiment, viral concentration was fixed, while the volume of viral particles was increased. Three different volumes of droplet were tested, from 2 μL to 30 μL. After 15 min on fingers, the proportion of recoverable infectious virus directly correlated with the size of the droplet (Fig. 3). After 15 min, 9/12 fingers contaminated with 30-μL drops were still infectious (all four individuals), whereas these proportions dropped to 6/12 fingers (2/4 individuals) for the 5-μL contaminant drop, and 2/12 (1/4 individuals) for the 2-μL drop. In a second experiment, the number of viral particles was fixed and the volume and concentration of the inoculum adjusted as follows: 2 μL, 4 μL and 10 μL of a viral suspension at concentrations of 7×10^6, 3.5×10^6 and 1.4×10^6 $TCID_{50}$/mL, respectively. After 15 min, the number of fingers still harbouring infectious droplets was closely related in all conditions, with 12/18 for the 2-μL, 12/18 for 4-μL and 9/18 for 10-μL droplets (Fig. 4).

We hypothesized that any mechanical pressure that spreads the infectious droplet on the contaminated surface may have an impact on the duration of viral survival by disrupting its integrity and microenvironment. To study the influence of this effect, which would certainly occur under real-life conditions, 2-μL infectious droplets were deposited on fingertips for 1 and 5 min. As in previous experiments, half were preserved and the remaining droplets were disrupted and spread on the surface of each fingertip immediately after deposition. Results showed that viral droplets remained infectious at a higher rate when conserved intact than when disrupted (Fig. 5a,b). Similar to previous experiments [17], A (H3N2) (Fig. 5c) and A (H1N1) 2009 (Fig. 5d) contaminated droplets remained infectious on 18/18 fingers (6/6 individuals) after 1 min and on 10/18 and 8/18 fingers (5/6 and 4/6 individuals), respectively, after 5 min. In contrast, viral viability decreased dramatically when droplets of similar volumes were disintegrated and spread on fingertips: 5/18 and 11/18 fingers (4/6 and 5/6 individuals, respectively) were still infectious at 1 min, but 0/18 and 3/18 fingers (0/6 and 2/6 individuals, respectively) remained infectious after 5 min with A (H3N2) and A (H1N1) 2009 viruses, respectively.

Discussion

Our experimental study was designed to reproduce as far as possible conditions that might lead to human finger contamination by the influenza virus in the community. Fingertips of

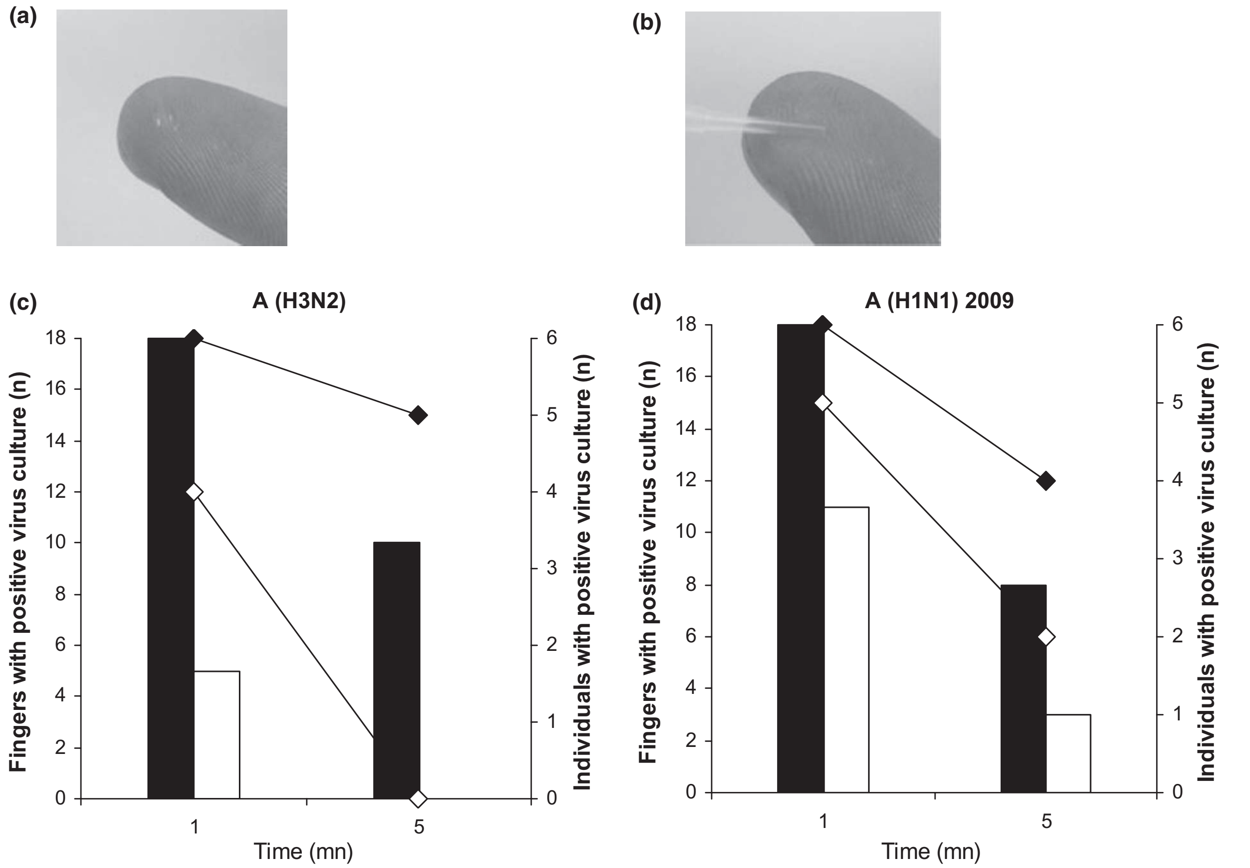

FIG 5. Impact of mechanical constraint on viral survival. (a) 2 μL of viral inoculum remained intact (black bars and diamonds in c and d) or (b) was disrupted immediately after deposition on fingers (white bars and diamonds in c and d). Survival of infectious viruses was evaluated by the number of fingers (bars) and individuals (diamonds) where infectious virus could be isolated 1 min and 5 min after deposition of influenza A (H3N2) (c) and A (H1N1) 2009 viral suspensions (d).

volunteers were contaminated with viral suspension mixed in human respiratory secretions at concentrations recoverable at the peak of symptoms during an influenza infection in children or adults [20,21]. The volume used (2 μL) was similar to large droplets that could be generated after sneezing and coughing. The fact that we used a respiratory mucus pool spiked with different influenza virus concentrations represents the best attempt to reproduce real-life conditions. When droplets were not subject to any mechanical constraints and their integrity preserved, it was possible to recover infectious influenza A (H3N2) and A (H1N1) 2009 viruses for a period of time extending up to 30 min. This is certainly long enough to provide opportunities for self-inoculation and possibly hand-to-hand transmission. A larger volume of droplets, as could occur in young children or during healthcare, would favour influenza virus stability and also in this way increase viral transmission. Our results are consistent with those of a previous study showing that hands contaminated with a non-physiological, highly concentrated viral suspension of 1 mL remained infectious at least 1 h at room temperature [17]. Of note, we showed that infectiousness decreased rapidly within 30 min following contamination.

Beyond the previously recognized beneficial effect of respiratory mucus for viral survival [10,12], we highlight that the size of the contaminant droplet and its physical integrity have a direct impact on the persistence of the infection. Virions contained in large 30-μL drops can be isolated in most cases after 15 min, compared with a minority of cases for small 2-μL drops containing the same viral concentration. Of note, a 30-μL volume is unlikely to be generated by respiratory secretions, but could be easily achieved in sputum. The question as to whether the higher volume of respiratory mucus directly conferred protection against external conditions (e.g. by preservation of a beneficial microenvironment) or if it is rather the higher absolute number of viral particles that have an impact on viral stability has been addressed by using droplets of different sizes, but containing the same number of particles. These experiments showed that the number of infectious virions contained in droplets is a key factor and possibly as important as the volume of droplets. The recent study by Grayson *et al.* [17] showed also that an inoculum of very high volume and viral concentration derived from cell supernatant, can lead to prolonged survival on hands, even outside a respiratory mucus environment. We showed also that viral infectiousness decreased within a few minutes when droplets were mechanically disrupted and spread on the skin surface, thus demonstrating that the presence of a protective microenvironment is important. Under real-life conditions, droplets present on fingers have a high risk of being disrupted or spread on the skin surface and this will undoubtedly rapidly decrease infectiousness. Based on our results and those of others [10–12,18], respiratory mucus has a protective role. Thus, skin and finger contamination from the respiratory tract or from environmental surfaces, subsequently followed by mucosal self-inoculation, is biologically plausible. To confirm this observation, hand washing was shown to decrease respiratory virus spread in schools [22] and influenza transmission in the household setting [23].

The risk of respiratory tract infection by contaminated fingers depends on several factors. One is the recovery rate of infectious virus from a contaminated surface. Another is the rate of contact between facial mucosa and contaminated hands. Previous studies involving human volunteers revealed an average of 15 hand-to-face contacts per hour [24]. Self-inoculation with the hand as intermediary has been demonstrated for rhinovirus infection [25]. For influenza, such studies have not been performed; such an analysis would require more sophisticated, controlled human infection studies. Our results nonetheless are compatible with this theorized mode of transmission.

We observed a slight difference between influenza A (H3N2) and A (H1N1) 2009 virus survival in our experiments. The latter appeared to be more resistant, but this needs to be confirmed given the limited number of observations. In animal models, the A (H1N1) 2009 virus appeared to transmit as efficiently as seasonal influenza viruses via direct contact [26], but differences between viral type and subtypes should be expected. Our experimental setting has several intrinsic limitations. The number of individuals and the diversity of influenza virus tested were limited; the viral concentration used was relatively high, particularly for the A (H3N2) virus; and respiratory droplets were reconstituted (but still using natural respiratory mucus) and artificially deposited on the skin surface. However, the reproducibility and the consistency of the experiments over time were excellent.

In summary, influenza A viruses have the potential to easily survive on the skin surface of hands. Infectiousness can be preserved as some individuals shed infectious particles for up to 30 min, even if the contaminant droplet was disrupted on the skin surface. This observation provides biological support to epidemiological observation [18–23] and is also consistent with recommendations to promote hand hygiene during influenza outbreaks [5], both in the community and the healthcare setting.

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Transparency Declaration

No potential conflict of interest exists.

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